Scientists grow heart tissue in Bioreactor

Results are a significant step forward in NASA-supported research

From an MIT press release and earlier Science@NASA stories

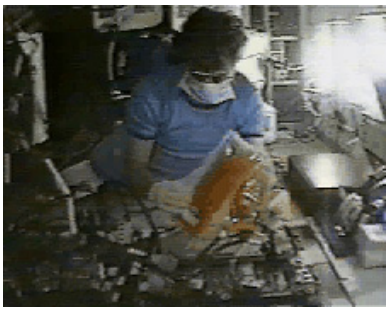
Oct. 5, 1999: If you've ever seen a pile of ivy that has taken the shape of an old barn that it has overgrown, you've seen the principle that researchers are following in trying to grow replacement parts for bodies. In research partly sponsored by NASA, scientists at the Massachusetts Institute of Technology have reported advances in characterizing the structural and electrical properties of heart tissue, and they've defined key parameters for growing the tissues.

Right: A kudzu-covered barn in North Carolina illustrates what MIT and other scientists are achieving in tissue engineering research: encouraging cells to grow in the shape of a structure such as a portion of damaged heart. Photo courtesy of Jack Anthony's Kudzu web site.

Their results are reported in the August issue of the *American Journal of Physiology - Heart and Circulatory Physiology* and the September issue of *Biotechnology and Bioengineering.*

The work is led by Dr. Lisa Freed, a principal research scientist in the Harvard-MIT Division of Health Sciences and Technology, working with Dr. Gordana Vunjak-Novakovic and other colleagues at MIT, Harvard Medical School, Boston University, and Brigham and Women's Hospital.

Their work is supported by NASA's Biotechnology Cell Science Program, directed by Dr. Neal Pellis at NASA's Johnson Space Center. the NASA program involves more than 100 scientists, engineers, and support personnel around the nation. A series of experiments has been carried out aboard the Space Shuttle and Russia's Mir space station, and soon will be expanded aboard the International Space Station. The Cell Biology Program is managed by NASA's Microgravity Research Program at Marshall Space Flight Center in Huntsville, Ala.



Left: Astronaut John Blaha operates the Bioreactor aboard Mir. Photo credit: NASA/Johnson.

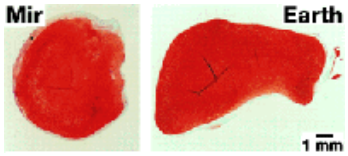
The Bioreactor was developed by NASA to simulate the weightless environment of space by putting cells in a growth medium that constantly rotates and keeps the cells in endless free-fall.

For many people, culturing cells means putting some small number into nutrient media in a dish or a tube and letting them grow. However, this kind of approach does not provide the culture environment that supports tissue assemblies. Without a proper 3-D assembly, epithelial cells (the basic cells that differentiate tissue into specific organ functions) lack the proper clues for growing into the variety of cells that make up a particular tissue.

In a rotating Bioreactor, the cells can be fooled into thinking they are in a body. With a plastic lattice to help direct their growth, cells can be encouraged to grow in predefined shapes, just as the vine-covered barn gives shape to vines.

Between September 1996 and January 1997, Freed and Vunjak-Novakovic with NASA colleagues achieved the first such results when they grew cartilage aboard the Space Station Mir in the first tissue-engineering experiment in space. They published their results in the December 1997 issue of the *Proceedings of the National Academy of Sciences* . This followed Freed's first successful experiment in engineering heart tissue: the cells she had "seeded" on a three-dimensional scaffold outside a living body began beating as one. "It was my most awesome laboratory moment ever. No one had ever done this before," said Freed. That work was completed in 1994 and published in 1997.

The MIT work is key to engineering three-dimensional cardiac tissue that could eventually be used to repair damaged heart tissue inside the body, test new drugs, and study general cardiac tissue development and function. Although it could theoretically lead to the creation of an entire heart, the researchers stress that substantial problems must be solved before that could happen. For example, while the current constructs resemble heart muscle, they lack blood vessels.



Left: Engineered cartilage samples grown as part of Freed's earlier research with Bioreactor aboard Mir. Photos: ***Proceedings of the National Academy of Sciences*** .

"We've developed one component, but that is only the first step," Freed said.

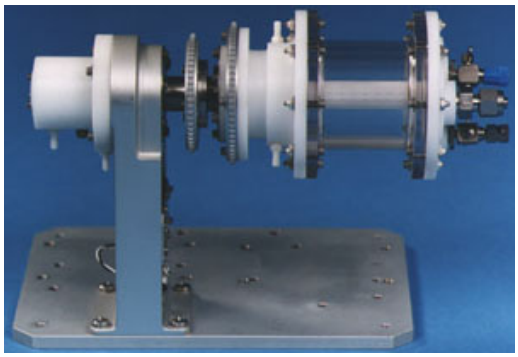
The MIT approach involves seeding cardiac cells onto a 3D polymer scaffold that slowly biodegrades as the cells develop into a full tissue. The researchers have used the same technique to grow other tissues .

The cardiac cells are cultivated on scaffolds 5 mm in diameter by 2 mm thick. The cell/scaffold constructs are placed in a rotating bioreactor that supplies the cells with nutrients and gases and removes wastes.

"The bioreactor is a kind of microenvironment that gives cells the signals they would ordinarily see in the body," said Vunjak-Novakovic. "This overall system allows us to study specific effects of the cells, scaffold, and regulatory signals on tissue development and function," Dr. Freed said.

Turning a problem on its side

It has long been established that cells and tissue growing in microgravity - the weightless condition obtained in space - can grow and mutate in ways different than on Earth. A perpetual challenge for the experimental study of these phenomena has been simulating the conditions of space so that complete laboratory studies can be done by numerous investigators on Earth. The simulated growth of mammalian cells in tissue culture needed to duplicate the quiet conditions of orbital free-fall in a way that allowed for maintaining fresh media and oxygenation.



Left: A ground-test model of the NASA Bioreactor shows the key element, a rotating plastic cylinder enclosing a tubular membrane that infuses growth media and oxygen and removes wastes. Credit: NASA/Johnson Space Center.

To solve the problem, NASA in the 1980s developed the bioreactor, a can-like vessel equipped with a membrane for gas exchange and ports for media exchange and sampling. As the bioreactor turns, the cells continually fall through the medium yet never hit bottom. Under these quiet conditions, the cells "self assemble" to form clusters that sometimes grow and differentiate much as they would in the body. Eventually, on Earth, the clusters become too large to fall slowly and research has to be continued in the true weightlessness of space.

It has been well established that a number of cell types grow in the bioreactor on Earth for extended periods in ways that resemble tissue-like behavior. For this reason, the bioreactor also provides cell culture studies with a new tool for the study of 3-dimensional cell growth and differentiation.

Bioreactors have been used aboard the Mir space station to grow larger cultures than even terrestrial Bioreactors can support. Several cancer types, including breast and colon cancer cells, have been studied in this manner. Continued research using the NASA Bioreactor is planned aboard the International Space Station.

Advances in engineering heart tissue reported by MIT scientists, colleagues. Original MIT press release. Sept. 28, 1999.
Bioreactor expands health research. Sept. 8, 1998.
NASA device gives new dimension to cell science.
NASA using space incubator to understand breast cancer. Oct. 1, 1998. Bioreactor research could help women's health on Earth and in space

In the *American Journal of Physiology* , the team characterized the structure and electrical properties of the cardiac constructs grown in conventional spinner flasks. Using a custom-designed electrode array, they applied electrical signals to the tissues and got them beating. They then studied parameters associated with impulse propagation through the tissue. For example, they found that constructs conducted electrical impulses half as fast as tissues grown in an animal's body.

In *Biotechnology and Bioengineering* , the team described how parameters like cell density, cell source (neonatal rat or chick embryo), and different cultivation conditions affect tissue growth. This work included the NASA Bioreactor and conventional spinner flasks.

"We've identified a set of conditions that so far appear to be best for cardiac tissue engineering," Vunjak-Novakovic said.

Work continues.

Understanding the structure of cancer

A different sort of tissue engineering - understanding the growth and spread of breast cancer - also is being addressed with the Bioreactror.

"The type of mammary cells we are growing comes from an individual susceptible to breast cancer, and that susceptibility is likely driven by damage caused by ionizing radiation," said Dr. Robert Richmond, director of the Radiation and Cell Biology Laboratory at NASA's Marshall Space Flight Center in Huntsville, Ala. Space exploration will involve slightly increased exposures of crew members to radiation, so what we learn from these cells could help help justify methods of female crew selection, and help manage breast cancer in the national population at the same time."

Right: Richmond extracts some breast cells from a liquid nitrogen dewar storing about half of the cell collection (the other half is in a separate dewar). In the right background are two incubators, one holding cells in conventional dishes (top) and one holding short bioreactors (bottom). Photo credit: Dennis Olive, NASA/Marshall.

He is conducting experiments that will determine if 3-dimensional constructs of normal breast tissue in the bioreactor will respond to estrogen. If so, then Bioreactors could be used to tailor hormonal therapies that more closely match what will stop growth of cancer cells with minimal side effects for the patient.

Richmond is using a cell repository from noncancerous breast tissue donated by a young woman carrying a single defective ATM gene. The debilitating syndrome ataxia-telangiectasia (A-T) results when both of the two ATM genes normally present in cells of the body become defective. These A-T individuals have about a 100-fold increased risk of all cancers plus other serious problems. Women carrying only one defective ATM gene are clinically normal, but have about a 5-fold increased risk, or susceptibility, to breast cancer. To reduce her breast cancer risk to near-zero, the donor elected to have a double mastectomy.

Her breast tissues now reside in a cell bank as perfectly matched cell types - preserved in liquid nitrogen - that will allow experimental results of today to be compared with experimental results obtained for many years to come.

In the bioreactor, these cells will grow in normal fashion because they are normal except for the single defective ATM gene. Once the normal tissue-equivalent model is defined, then these same cells can be manipulated to mimic the stages of breast cancer formation, and the model-related differences evaluated.

"We have grown noncancerous human breast cells in the NASA Bioreactor," Richmond said. "Our observations suggest there is much to learn and value to be gained from the study of their tissue-equivalent growth."

"There are substantial problems that must be addressed before we could use these tissues for, say, repair of heart defects inside the body," Freed said.

In addition, constructs must be bigger, stronger, and made of human rather than animal cells that have been modified so they will not be rejected by a recipient.

The first successful experiment five years ago "showed that cardiac tissue engineering was possible," Vunjak-Novakovic said. The current papers are the first to quantitatively characterize tissue properties.

"They're really the beginning," she concluded.

"Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies." American Journal of Physiology, Heart and Circulatory Physiology. Vol. 277, Issue 2, H433-H444, August 1999. **N. Bursac[1,2], M. Papadaki[1], R. J. Cohen[1], F. J. Schoen[3], S. R. Eisenberg[2], R. Carrier[1], G. Vunjak-Novakovic[1], and L. E. Freed[1]** [1] Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge 02139; [2] Department of Biomedical Engineering, Boston University, Boston 02215; and [3] Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115

The objective of this study was to establish a three-dimensional (3-D) in vitro model system of cardiac muscle for electrophysiological studies. Primary neonatal rat ventricular cells containing lower or higher fractions of cardiac myocytes were cultured on polymeric scaffolds in bioreactors to form regular or enriched cardiac muscle constructs, respectively. After 1 wk, all constructs contained a peripheral tissue-like region (50-70 μm thick) in which differentiated cardiac myocytes were organized in multiple layers in a 3-D configuration. Indexes of cell size (protein/DNA) and metabolic activity (tetrazolium conversion/DNA) were similar for constructs and neonatal rat ventricles. Electrophysiological studies conducted using a linear array of extracellular electrodes showed that the peripheral region of constructs exhibited relatively homogeneous electrical properties and sustained macroscopically continuous impulse propagation on a centimeter-size scale. Electrophysiological properties of enriched constructs were superior to those of regular constructs but inferior o those of native ventricles. These results demonstrate that 3-D cardiac muscle constructs can be engineered with cardiac-specific structural and electrophysiological properties and used for in vitro impulse propagation studies.

"Cardiac Tissue Engineering: cell seeding, cultivation parameters, and tissue construct characterization." ***Biotechnology and Bioengineering*** , 64: 580-589, September 1999. R. Carrier, M. Papadaki, Maria Rupnick, F. J. Schoen, N. Bursac, Robert S. Langer, L. E. Freed, G. Vunjak-Novakovic.